SCIENTIFIC REPORT

The severe acute respiratory syndrome coronavirus in tears

S-C Loon, S C B Teoh, L L E Oon, S-Y Se-Thoe, A-E Ling, Y-S Leo

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Background: Severe acute respiratory syndrome (SARS) is a new infectious disease that caused a global outbreak in 2003. Research has shown that it is caused by a novel coronavirus. A series of cases is reported where polymerase chain reaction (PCR) testing on tears had demonstrated the presence of the virus. Detection of ocular infection from tears using the PCR technique has been widely used by ophthalmologists to diagnose infections for other viruses.

Methods: This is a case series report from cases classified as probable or suspect SARS cases. Tear samples were collected from 36 consecutive patients who were suspected of having SARS in Singapore over a period of 12 days (7–18 April 2003), and analysed by PCR using protocols developed by the WHO network of laboratories.

Results: Three patients with probable SARS (one female and two male patients) had positive results from their tear samples. Tear samples were used to confirm SARS in the female patient, who was positive only from her tears. The positive specimens were found in cases sampled early in their course of infection.

Conclusions: This is the first case series reported with the detection of the SARS coronavirus from tears, and has important implications for the practice of ophthalmology and medicine. The ability to detect and isolate the virus in the early phase of the disease may be an important diagnostic tool for future patients and tear sampling is both simple and easily repeatable. Many healthcare workers are in close proximity to the eyes of patients and this may be a source of spread among healthcare workers and inoculating patients. Ophthalmic practices may need to change as more stringent barrier methods, appropriate quarantine, and isolation measures are vital when managing patients with SARS.

Severe acute respiratory syndrome (SARS) is a recent disease that had a significant worldwide impact both in mortality and economic morbidity. Initial reports established from blood and respiratory samples show that the disease is caused by a novel strain of coronavirus.[1 2] The natural course of the disease has been shown to have an incubation period of 2–7 days, preceded by a prodrome of pyrexia (38.0℃ or more) and may be associated with chills, rigors, malaise, myalgia, dry cough, dyspnoea, or headache.[3]

Singapore was one of the countries adversely affected by SARS. The index patients presented in early March 2003 and by the end of the outbreak on 31 May 2003, 238 probable cases were diagnosed. In an effort to identify patients early at a time when diagnostic kits were still under investigation around the world, we collected specimens from various secretions, including tears from patients diagnosed with SARS.[3] Detection of ocular infection from tears using the polymerase chain reaction (PCR) technique has been widely used by ophthalmologists to diagnose infections especially of the herpesviridae family, such as the herpes simplex virus types 1 and 2,[4–6] Epstein-Barr virus,[7] varicella zoster virus, and human herpes virus 6.[8] Other viruses that been identified and genotyped from tears with this technique include hepatitis C,[9–11] hepatitis B,[12] measles,[13] and adenovirus.[14] Tears PCR has even been used to diagnose *Acanthamoeba* keratitis,[15] an infection which often poses diagnostic dilemmas to the ophthalmologist.

Finding the SARS coronavirus in tears is important, as the latter may be a potential source of spread. This is the first recorded case series of detection of the SARS coronavirus from tears.

METHODS

Patients who presented with symptoms of SARS from the period 11–18 April 2003 at Tan Tock Seng Hospital were included in the study. Probable and suspect cases were defined according to WHO case definitions.[16] Suspect patients were those presenting with fever of more than 38.0℃, respiratory symptoms and history of contact with a case of probable SARS, or were in an affected institution for which alternative diagnoses had been excluded. Probable cases were suspect cases with chest radiograph changes compatible with pneumonia or acute respiratory distress syndrome.

Patients were identified for sample collection once they met the case definitions for probable and suspect SARS. The patient's signed consent was obtained for each sample collection. Tear samples were collected by conjunctival swab technique. Eyelids were everted and samples were obtained by sweeping the inferior fornices of both eyes with sterile cotton tipped swabs without topical anaesthesia. The tips of the swab sticks were broken off and placed into Hank's balanced salt solution. Gloves were changed when collecting specimens in between patients to minimise risk of contamination. Samples were transported in ice to the WHO network laboratory in the virology section of the Department of Pathology at the Singapore General Hospital.

Reverse transcription-polymerase chain reaction

All samples were subject to RNA extraction followed by conventional qualitative reverse transcription-polymerase chain reaction (RT-PCR) with two sets of primers. The primers were targeted at different regions of the polymerase (pol) gene of the SARS coronavirus, which is one of the most conserved sites of the virus genome. The RT-PCR protocols were developed by the WHO network of laboratories[17] and are summarised in table 1. RNA extraction was carried out using the viral RNA kit (QIAamp, Qiagen, Australia) from 200 µl of the Hank's solution in which the swab was immersed. A volume of 6 µl of extracted RNA was used to generate cDNA using SuperScript II RNase H-reverse transcriptase (Invitrogen). A 20 µl reaction volume of RT mix (containing 6 µl viral RNA extract, 250 ng of random primers (PdN6),

Abbreviations: PCR, polymerase chain reaction; SARS, severe acute respiratory syndrome

250 mM dNTP mix, 0.01M DTT, 40 units ribonuclease inhibitor, and 200 units SuperScript II RT Rnase in 1X first strand buffer) was prepared. The RT mix was then incubated at 42℃ for 50 minutes and 70℃ for 15 minutes. The cDNA generated was subjected to PCR using two sets of primers. The PCR products were then analysed using a 2% agarose gel.

A positive result with either set of primers on the first round of RT-PCR had to be confirmed by re-extracting the RNA from the original sample and subjecting the sample to a second round of RT-PCR with both sets of primers. The result would be reported as positive in these circumstances: (1) if a positive result is obtained with both sets of primers, or (2) if one set of primers is positive and there was a positive PCR result from another sample type from the same patient.

Results and patient data were tabulated on an Excel worksheet and analysed using SPSS statistical software (v11.0) (Chicago, IL, USA). Differences in means were compared using the Student's *t* test. p Values of less than 0.05 were considered statistically significant.

RESULTS

Samples were collected from 36 patients initially suspected to have SARS over a period of 12 days (7–18 April 2003), 17 of whom were male and 19 were female patients. The majority were healthcare workers and female nurses. Using WHO case definitions, eight patients were eventually proved to be probable SARS (later confirmed by serology) and the other 28 were SARS suspects. Of the eight probable SARS, there were four males and four females. Mean age was 48.6 (SD 21.8) years (range 25–85 years, males 61.5 (22.9) years, females 35.8 (11.7) years). Tear samples from three probable SARS patients (37.5%) yielded positive PCR results, while none of the SARS suspect patients had a positive PCR result. Table 2 gives the profile of the three cases with SARS coronavirus RNA detected in their tears. Two were elderly male patients with multiple co-morbidities, while the third was a young female healthcare worker who had the virus detected only in her tears by current PCR kits.

All three positive cases had their tears sampled in the early phase (within 9 days of onset) of their illness (mean 4.0 (3.2) days). The other five probable SARS cases who had negative yield were sampled in the later part of their illness (mean 19.4 (11.2) days), although the difference did not reach statistical significance (p = 0.08).

DISCUSSION

In our small series, we have demonstrated the presence of viral RNA in the tears. Ophthalmologists examine patients at close distances and inadvertent physical contact with patients' eyes is inevitable. This is a potential hazard to healthcare workers in close contact with the face and specifically the eyes of SARS patients. There is a potential possibility of transmission to other patients through the use of reusable eye equipment such as the Goldmann applanation tonometer, trial contact lenses, trial frames, and even reusable pinhole devices which come in close contact with the patient's eyes. Our hands may also be a means of inoculating other patients. Splashing of infected body fluids onto the eyes has been a documented mode of infection even for viruses that are not usually spread by mere contact—for example, hepatitis B.[18] Thus, stringent barrier methods using the ''M3G'' (mask, gown, gloves, and goggles) should be the gold standard when dealing with suspected SARS patients. Increased vigilance to prevent spread to other eye patients through thorough meticulous disinfection of eye equipment is of crucial importance in breaking the chain of transmission as is maintaining strict hygiene of our routine outpatient procedures and practices.

The detection of the viral RNA by PCR may be a useful adjunct in early diagnosis of SARS and this may have important applications in commencing early treatment and appropriate supportive measures which may have better prognosis for the patient, as well as the institution of appropriate quarantine measures for contacts of this case. Moreover, tear sampling can be performed easily, painlessly, and repeatedly on the slit lamp or by the bedside without discomfort to the patient.

Our study was limited by a small sample size. Ophthalmologists may be a better choice of personnel in the collection of specimens as they are more familiar with tear sampling. Although precautions were taken, contamination from the upper respiratory tract cannot be ruled out. The negative results in the other cases may be due to differences in volume of fluid collected, the collection technique, or the

Table 1 PCR primers, reagent concentrations, and thermal cycling conditions used

Primer (source)	Primer sequence	Expected fragment size	PCR reagent formulation*	Thermal cycling profile
Sars1S/As (Germany)	Sars1S 5′-cctctcttgttcttgctcgca-3′	121 bp	5 μl 10X PCR buffer 1.5 mM $MgCl_2$ 250 mM of each dNTP 20 μM each primer	Hold: 95°C/3 min 10 cycles: 95°C/10 s 58°C/10 s
	Sar1AS 5′-tatagtgagccgccacacatg-3′		2 units of Taq polymerase 2 μl of cDNA 50 μl total volume	72°C/20 s 40 cycles: 95°C/10 s 56°C/10 s 72°C/20 s Hold: 72°C/5 min
Cor1/2 (Hong Kong)	Cor1 5′-caccgtttctacaggttagctaacga-3′	310 bp		Hold: 94°C/2 min 10 cycles: 94°C/30 s 56°C/60 s
	Cor2 5′-aaatgtttacgcacggtaagcgtaaaa-3′			72°C/45 s 35 cycles: 94°C/30 s 60°C/45 s 70°C/45 s Hold: 72°C/5 min

*Reagent was from Platinum Taq DNA Polymerase (Invitrogen).

Table 2 Profile of cases with PCR test positive for SARS coronavirus

	Case		
	1	**2**	**3**
Age	30	74	85
Sex	F	M	M
Details of admission			
Onset	8 April 03	2 April 03	14 April 03
Date of admission	9 April 03	7 April 03	14 April 03
Date of first abnormal CXR	14 April 03	11 April 03	14 April 03
Outcome	Discharged well	Dead	Dead
Date of death or discharge	23 April 03	23 April 03	18 April 03
Remarks	Healthcare worker infected by case 2	Inpatient from 29 March 03 for subacute IO	Inpatient for cholecystitis. Infected by sibling of the index case in SGH
Key laboratory indicators			
Total white count ($4.0–10.0\times10^9$/l)	8.0	4.0	6.5
Lymphocyte count (18–43%)	18.9	12.0	4.4
Platelet count ($160–340\times10^9$/l)	275	144	82
Lactose dehydrogenase (200–500 U/l)	283	540	955
SARS virus RNA from			
Tears	Positive	Positive	Positive
Stools	Negative	*Positive*	Positive
Nasal aspirate	Not sent	Not sent	*Positive*
Date of collection of tears for PCR	11 April 03	11 April 03	18 April 03
Day of collection from onset of fever	Day 3	Day 9	Day 4

timing of sampling. We propose the use of microcapillary pipettes or Schirmer's filter paper strips that allow collection of cellular material for electron microscopy and immunofluorescence.

We should also determine if the virus is also present in convalescent patients and to determine the infectiousness of tears. As all three positive cases had sampling performed early in the course of their illness, we hypothesise that the secretion of virus in tears occurs only during the early phase of this disease. Even as the epidemic has died down, we are warned of future outbreaks. This may be a simple tool in identifying probable cases in future and prospective trials are being designed for this purpose.

This study also suggests that SARS, like other viruses, can involve the eyes. An ocular review of convalescent probable SARS patients will show if this coronavirus causes any long term abnormalities and pathology to the ocular surface and retina. Results will be reported in due course.

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Authors' affiliations

S-C Loon, S C B Teoh, The Eye Institute, Tan Tock Seng Hospital, 11 Jalan Tan Tock Seng, Singapore 308433, Republic of Singapore
H-N Leong, Department of Infectious Disease, Singapore General Hospital, Outram Road, Singapore 169608
L L E Oon, S-Y Se-Thoe, A-E Ling, Department of Pathology, Virology Section, Blk 8, Singapore General Hospital, Outram Road, Singapore 169608
Y-S Leo, Centre for Communicable Diseases, Tan Tock Seng Hospital, 11 Jalan Tan Tock Seng, Singapore 308433

Correspondence to: Dr S-C Loon, Department of Ophthalmology, 5 Lower Kent Ridge Road, Main building Level 3, National University Hospital, Singapore 119074; ploonsc@yahoo.com

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